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Part II

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National Institutes of Health

Program To Assess Risks of Recombinant DNA Research; Proposed First Annual Update

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Program To Assess the Risks of Recombinant DNA Research: Proposed First Annual Update

AGENCY: National Institutes of Health.

ACTION: Notice of proposed first annual update of a program to assess the risks of recombinant DNA research.

SUMMARY: On September 13, 1979 the initial plan was published in the **Federal Register.** This notice sets forth a proposed first annual update of the program to assess the risks of recombinant DNA research. Interested parties are invited to submit comments concerning the plan. After consideration of those comments and comments by the NIH Recombinant DNA Advisory Committee, the Director of the National Institutes of Health will publish the final update in the **Federal Register.**

DATE: Comments must be received by December 16, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Chief, Office of Specialized Research and Facilities, NIAID, Building 31, Room 7A04, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Additional information may be obtained from Dr. John Nutter, Chief, Office of Specialized Research and Facilities, NIAID, National Institutes of Health, Bethesda, Maryland 20205 (301–496–5643).

SUPPLEMENTARY INFORMATION:

I. Introduction

With the issuance in December 1978 of revised guidelines for the conduct of recombinant DNA research, the Secretary DHEW requested that the National Institutes of Health (NIH) prepare an NIH Risk Assessment Plan which after review by the Recombinant DNA Advisory Committee (RAC) and publication in the **Federal Register** for comment, would be made final and updated annually. The present document is the initial response to the request for an annual update.

II. Scientific Aspects

We stated in the Final Plan and it is still our conviction that:

"The vast majority of information relevant to recombinant DNA risk analysis has already come from research not primarily designed to provide information on risk. This will undoubtedly continue to be the case. This information will be obtained chiefly from publications in the scientific literature, from persons with special scientific knowledge, and from ongoing basic biomedical research. Risk assessment analysis will require continuing review of data developed in the fields of microbiology, infectious diseases, and related biological research.

Some essential information has been, and will continue to be, derived from projects specifically designed to assess various aspects of potential risks associated with recombinant DNA experimentation. Such experiments will be supported by the Intramural and the Extramural programs of NIH. Many experiments may also be conducted in the private sector or may be funded by other agencies or governments.

The essential goal of a successful risk assessment plan will be the development of means to collect, collate, coordinate, evaluate, and disseminate data obtained from all sources."

The Scientific Aspects of the Plan noted that a number of events must occur before a laboratory microorganism becomes a possible risk to people or higher organisms outside the immediate laboratory environment. A major aspect of the risk assessment plan was to acquire and analyze information and data relevant to those elements for the three general categories of host-vector systems in use; prokaryotic, lower eukaryotic and higher eukaryotic systems. Initial emphasis was on the prokaryotic *E. coli* K–12 systems because those were, and remain, the systems predominately used by investigators and because needed areas of investigation had already been identified. Seven areas were identified as requiring particular consideration and progress has been made in collecting and/or analyzing data for all of them. Before considering these it is worth saying that, despite intensive study by the RAC Subcommittee on Risk Assessment and NIH staff, several conferences and workshops to consider specific issues and several experiments, no risks of recombinant DNA research have been identified that are not inherent in the microbiological and biochemical methodology used in such research. A synoptic report of progress follows and all data, reports, and other documents referred to are available on request.

Prokaryotic Host-Vector Systems

1. Survival in the environment and the potential for selective advantage of organisms carrying recombinant DNA should they survive was cited as a matter of concern. NIAID had four contractors working on various aspects of this issue; the contracts were originally awarded to provide independent testing in the process whereby EK2 systems could possibly be elevated to EK3 status. The tests were performed in situations simulating accidential spills in the laboratory, in a model sewage treatment system, in mice and cultures simulating the mouse gastrointestinal system, and finally in germfree mice and humans. The status of the work of all four contractors, at the time the Plan was initially published, can be found in the Recombinant DNA Technical Bulletin, Vol. 2 No. 2, July 1979. The expiration date of all contracts has now been reached and Final Reports are or soon will be available.

a. The work of the contractor testing in "accidental spill situations" was completed shortly after publication of the Plan and was in two areas: (1) to determine whether selected strains of *E. coli,* used as hosts to propagate recombinant-DNA molecules, and some phage and phasmid vectors would survive or retain their capacity to infect or transform host cells after exposure to the environments expected to occur in the event of spill or aerosolization, (2) to inventory and simulate common procedures used in recombinant DNA laboratory studies so that aerosol output potential of the procedures could be accessed.

Considerable effort was expended on quantitative descriptions of the biological characteristics of hosts and vectors of concern in current rDNA studies and the physical disperson aspects have been examined with particular attention to procedures unique to such laboratory work.

The data on biological survival characteristics are applicable to evaluation of potential dosage to workers or to the environments both from accidents creating massive release and from the continuing releases incidental to the laboratory procedures. Evaluation of the effect of survival of the agents in the course of routine work should assist in prudent and cost-effective application of physical and biological containment. Further, it can lend emphasis to the areas in which training in "good practice" can be most productive.

b. The Survival of EK1 and EK2 Systems in Sewage Treatment Plant Models.

In an early series of studies utilizing bench scale models of wastewater treatment facilities, *E. coli* DP50supF, *E. coli* Chi 1776 and phage Charon 4A concentrations were shown to be reduced by at least two orders of magnitude by a conventional treatment chain (including primary settling

followed by activated sludge treatment and anaerobic digestion of all sludges generated). This work was described in brief form in the *Recombinant DNA Technical Bulletin* of July 1979.

Similar results are now available for the survival of *E. Coli* Chi 2656 (which carries plasmid pB322) and for *E. Coli* GF 2174 (carrying plasmid pBR325). Evidence also was sought in these studies for transfer of the plasmids to indegenous coliforms and, within the sensitivity of the methods used, such transfer was not demonstrated. Experiments favoring plasmid transfer in raw wastewater and in primary biological sludges also failed to demonstrate such mobilization.

Likewise, *E. Coli* 2e01C carrying a lambda prophage (λcI857 nin5 plac5) was removed effectively by wastewater treatment; no free lambda phage was observed at any point in the treatment train.

Studies have been completed measuring the survival of an indigenous *E. Coli* and *E. Coli* K–12 in these treatment plant models as well. The results of experiments utilizing such genetically tagged organisms confirm an earlier report that conventional domestic wastewater treatment processes will result in at least two $\log_{10}$ reductions in concentration of indigenous, EK1, and EK2 organisms. These results are similar to those obtained with the indigenous wastewater microflora. Further reduction of these host-vector populations will depend on appropriate sludge treatment and effluent disinfection.

c. The contractor who was performing tests in mice and in cultures made significant progress during the intervening year. The most notable result was the establishment of a mathematical model to study the exchange of plasmids between normal hosts and indigenous bacteria.

Plasmid transfer in continuous flow (CF) cultures of defined or natural intestinal flora occurred with similar efficiency as in pure cultures in vitro. One can conclude that the capacity of *E. coli* K–12 strains to function as plasmid donors or recipients was not impaired by the presence of an indigenous microflora. *E. coli* Chi 1776 donated plasmid R1drd19 with somewhat lower efficiency than a standard *E. coli* K–12 donor.

Plasmid transfer in mice harboring a defined intestinal microflora appears to have the same degree of efficiency as in the CF cultures, because the rate constants calculated on the basis of the equations are similar to those obtained in CF cultures. However, the validity of this mathematical treatment for mice is not completely certain, because the mathematical model is based on the assumption that the bacteria are freely suspended, which is not likely to be the case in the animal. Nevertheless, the data suggest that plasmid transfer efficiency in the gut should not differ profoundly from that in CF cultures.

These conclusions are based on similarities of transfer rate constants as measures of donor and recipient capacity. The actual rates of transfer are, of course, critically dependent on the concentrations of donors and recipients. The *E. coli* populations in normal mice (and in people) are so low (approx. 10^5 per ml gut content), that little plasmid transfer occurred in the mice after the initial 20 hours of the experiment, i.e. after the large inoculum of donors had passed through the animals. This was true in spite of the fact that the resident *E. coli* were artifically implanted, highly efficient recipients for the highly efficient conjugative plasmid. It is not very common to find *E. coli* of such high recipient capacity in natural gut flora. Despite the fact that the capacities of the *E. coli* strains to donate or receive the plasmid were not seriously impaired in the gut, the quantitative parameters in the normal gut are such that little actual transfer occurred once the large initial inoculum of donors had been eliminated.

From these data one may tentatively conclude that the probability for triparental transfer of a non-cojugative plasmid in the normal gut would be exceedingly small. The only circumstances in which one could imagine a realistic possibility for triparental transfer to occur, would be at times when abnormally high *E. coli* populations are present in the gut, as may be the case during fasting and other types of stress, in diarrhea or as a consequence of antibiotic therapy.

d. The major effort of the contractor who was testing in both mice and humans in the interim has been to initiate triparental mating studies using Chi 1666 (an EK1 host), the plasmid pBR322 and two additional mobilizing plasmids.

The studies are designed to determine if the presence of a mobilizing plasmid in a bacterial host cell can cause the transfer of the non-conjugative pBR322 to the indigenous microflora of either mice or humans.

Two studies have been done in mice. In both cases germ-free mice were colonized with human *E. coli* to provide an array of potential recipients and then fed the Chi 1666 containing the three plasmids. In the first study $5x10^9$ organisms were fed once and fecal samples from the mice were pooled to facilitate assay; Chi 1666 could only be isolated during the first day of the study. In the second mouse study, the Chi 1666 containing the plasmids were fed daily for 4 days to the mice and their drinking water contained tetracycline. In this latter group the Chi 1666 survived at levels of 10^2/gram or less for the 4 days. In neither case, however, was the pBR322 mobilized to the indigenous flora.

Finally, Chi 1666 containing pBR322 and the two mobilizing plasmids was fed to 4 human subjects. More than 70 fecal samples were collected during the study and a large number of cultures and subcultures (2500) performed. The Chi 1666 survived from 3½ to 7 days and that interval corresponds well with previous data from studies in which Chi 1666 was fed to humans with the without pBR322. While the data for transfer of pBR322 await DNA:DNA colony hybridization and further confirmation, from the data already available on the two transferable plasmids, we can make certain estimates. Highly transferable pSL222–4 was found to transfer to coliforms at a frequency no higher than $8x10^{-6}$. This value is 10^{-5} lower than seen when the plasmid is tested under the best laboratory conditions. Thus pBR322, which is transferable by F-like R plasmids at 10^{-5}–10^{-8} would be expected to be transferred at an extremely low frequency; probably below experimentally detectable levels. The same is presumably true of potential mobilization by the second conjugative plasmid, although the data for their mobilization of pBR322 are not readily available. We would estimate that pBR322 would be transferred at a frequency at least 10^4–10^5 fold less than the conjugatable plasmid, i.e., 10^{-9}–10^{-10}.

2. Progress on the evaluation of the transmission of vectors from *E. coli* K–12 to other bacteria in the gastrointestinal tract of animals and human beings has been made via three approaches.

a. Data reported in section 1 by two of the contractors relates directly to this question.

b. A meeting was convened on August 30, 1979 at the NIH with the purpose of considering Falmouth Workshop Protocols I and II (Jour. Infect. Dis. *157*, 704–708, 1978) and some related issues. Dr. Stanley Falkow served as Chairman and sixteen participants and NIH staff attended.

Protocol I addressed the colonization and transmission of plasmids from *E. coli* K–12 in the gastrointestinal tract of humans to other bacterial strains in the intestinal flora. The Working Group

unanimously recommended that the NIAID not initiate new studies to pursue the investigations as written in Protocol I. This judgment was based on a review of data that existed at the time of the Falmouth Workshop, a consideration of some newer published data and the results of contracts that NIAID was supporting. As written by the Falmouth Workshop Participants, the experiments were to be based on *E. coli* K–12 and this was judged to not be a fruitful experimental model. It was a clear consensus of the Group that, based on the available data, it can be predicted that only negative results will be obtained and that limited resources could be better expended in other pursuits.

Protocol II was designed to study the transmission of plasmids from *E. coli* K–12, including Chi 1776, into the normal intestinal flora utilizing a germ-free mouse model. The Working Group unanimously supported the view that the NIAID should not initiate new studies for the Protocol as it was written but to rely on the contracts to supply some additional data. This judgment was based on the same reasoning and data base considered for Protocol I.

During discussion of the issues cited above it was obvious that the Working Group felt that a more beneficial use of monies would be to support the training of workers in good microbiological laboratory practices and to support research aimed at gaining a better basic scientific understanding of bacterial colonization and plasmid mobilization. Toward this last goal the Working Group strongly recommended that the NIAID support studies that could obtain quantitative data, expand scientific knowledge in an important area and which may prove useful at some future date for risk assessment. The Group felt that such studies should be performed directly in humans and employ wild type *E. coli* (not K–12). Strain HS containing pBR325 was suggested as a good initial combination and the study should be developed to assay for both survival and transfer to the indigenous flora. [These studies are now in their early stages and being performed by an NIAID contractor at the University of Maryland Medical School.]

The full transcript of this meeting and a verbal report was made to RAC at their March 1980 meeting and the recommendations were approved by that Committee.

c. NIAID identified two relevant grant applications and the National Advisory Allergy and Infectious Diseases Council supported selective payment of these projects for inclusion into the risk assessment program.

One grantee will focus on the mechanisms that control human and animal gut flora. Of the four stated proposed aims of the research plan three relate to issues of importance to the NIH Recombinant DNA Risk Assessment Program. They are: (1) characterize and extend the application of anaerobic continuous flow cultures, (2) analyze the efficiency of plasmid and bacteriophage transfer, and (3) determine whether human microflora can be maintained in gnotobiotic mice and in anaerobic continuous flow cultures. The issue of mobilization of vector plasmids to the indigenous flora has always been a concern when considering the use of *E. coli* K–12 based host-vector systems. Most recently concern has been expressed over the potential for exchange of plasmids between the Enterobacteriaceae and the anaerobic flora, principally members of the genus Bacteroides. This project has the capacity for filling a significant void in experimental data.

A second grantee will be exploring a related issue which focuses on the molecular mechanisms of *E. coli* colonization of the intestine, specifically on the relative importance of plasmid or chromosomal determinants of colonization.

At this point in time the majority of experiments using recombinant DNA technology employ host-vector systems based on *E. coli* K–12 and its plasmids or bacteriophages. Prominent among the scenarios raised early in the debate over use of this technology was the possible colonization of the intestinal tract by host-vector systems followed by various consequences depending on the elaboration of a product which would cause harm to the individual by either direct or indirect mechanisms. There now are a considerable number of studies describing the survival of various types of *E. coli* in the intestinal tracts of man and mice and they demonstrate a tremendous disparity in the survivability and colonization potential of such strains. A complete understanding of those factors that control survival and colonization may permit the development of both safer and more useful *E. coli* hosts in the future as well as perhaps provide data suggesting adjustments in the physical containment requirements of the NIH Guidelines governing use of this technology.

3. Experiments testing *E. coli* K–12 host-vector systems carrying recombinant DNA for virulence have been done by NIH scientists. The studies were designed to determine the pathogenicity and stability of shotgun clones of Saccharomyces DNA when used with both plasmid and bacteriophage vectors. In this experimental model, which utilized mice, there was no evidence that the presence of segments of the entire yeast genome altered the inherently low pathogenicity of *E. coli* K–12 in any way.

4. A Workshop was convened to consider two areas [within the NIH Program] recommended by the Risk Assessment Subcommittee of RAC. This Workshop was designed to define the scientific issues and assess the potential risks of: (a) Possible direct adverse effects of hormone-producing strains of *E. coli* K–12, and (b) the possible occurrence of autoantibodies or autoreactive cells due to the production of eukaryotic polypeptides (including hormones) by *E. coli* K–12 should they colonize higher organisms.

In the charge to the Workshop it was noted that these potential risks were under consideration because there is still debate over the degree of possible risk, even though *E. coli* K–12 has apparently lost those known characteristics that are required for colonization of the normal intestinal tract.

The purpose was to decide whether the two specific possibilities are valid scientific hypotheses if such an event did occur; and if valid, to determine if sufficient experimental data already exists to make a final judgment concerning possible risk. If data were insufficient, the participants were asked to outline those types of studies necessary to develop the definitive information on these issues.

The meeting brought together 92 scientists from the fields of immunology, endocrinology, physiology, microbiology, infectious diseases and other appropriate disciplines. The Chairmen's Summary and a full transcript of the Final Plenary Session was distributed to all participants and also the RAC for their review at the June 1980 meeting. In addition to the report of the Director, NIAID, Drs. Setlow and Campbell prepared a separate report and the RAC Risk Assessment Subcommittee reported their analysis of the Workshop.

NIAID Plans in implementing the four prime recommendations was influenced by the RAC discussions. At this time we propose to respond to the recommendations as follows:

a. It was recommended that experiments of the folowing general type should be performed: "*E. coli* cells carrying recombinant NDA and making a mammalian protein are introduced into living animals under conditions where they cause genuine infection (e.g.

an abscess), with subsequent monitoring for breakdown of immune tolerance."

This subject stimulated the most discussion during the final plenary session of the Workshop. The sentiment was to acquire the data as a matter of scientific interest rather than to answer the risk assessment question. An RFP is being developed to solicit applications from interested investigators. Immunologists from the NIAID staff will design the experimental protocol for the workscope and other specifications of the study which is most likely to be an animal model system employing insulin. The matter however is still in the formative stage and proposers will have considerable leeway in outlinging plans to answer the primary questions.

b. Additional information was requested on the handling and absorption of polypeptides in normal and pathologic colon and the potential effects of synthetic peptides on the bowel itself.

We have confirmed that this is an issue about which there are little direct data. The Workshop participants opined that although their own calculations revealed that only extremely small amounts of hormones would be produced under the most ideal conditions, data should still be sought for potential future needs. The issue of the potential effects of synthetic peptides on the bowel itself is different from that of hormones and that discussion was centered on interferon and its direct effect on cells.

Although this will be a difficult study to initiate because of the technical difficulties as well as the process for approval of research in human subjects, NIAID is considering solicitation of grant proposals through an RFA. The study will have as it objective a determination of the fate of peptide hormones when deposited in the distal small intestine and large intestine of humans. These sites are relevant to the production of hormones by recombinant DNA technology because they represent the regions of colonization by *E. coli.*

c. It was suggested that additional information is needed on the potential transfer of plasmids to anaerobic bacteria from *E coli.* This recommendations differs from the findings of the ad hoc group convened in August 1979 which RAC reviewed at the March 1980 meeting. That expert Group noted that by studing the epidemiology of plasmids the conjugative plasmids of *E. coli* are not found within the anaerobic flora of the gut. The uniform use of non-conjugative plasmids in rDNA research further reduces the likelihood of transfer.

It is likely that the requested data will be forthcoming from NIH (NIAID) supported studies on plasmids through the regular grant-supported research programs and that new initiatives are not needed. In fact, the NIAID Advisory Council identified a grant application for selective payment that addressed some aspects of this issue; this action will initiate the acquistion of the data at an early date. There is no plan to develop a RFA at this time but NIAID staff will continue to identify all appropriate incoming applications as potential sources of data.

d. It was recommended that NIH communicate with the Center for Disease Control (CDC) about possible types of health surveillance for workers using recombinant DNA.

A CDC representative was present at the Pasadena meeting and discussed some approaches to this issue. Furthermore, representatives of the National Institute for Occupational Safety and Health and the Occupational Safety and Health Administration were also present and therefore, are cognizant of the Workshop recommendation. All of these agencies are members of The Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research which is discussing this issue.

5. NIH scientists have continued a further evaluation of the biological activity of polyoma virus DNA cloned in *E. coli* host vector systems. It had been reported (Science *203:* 883–892, 1979), when the Final Plan was initially published that polyoma DNA was noninfectious when cloned in EK2 plasmid and bacteriophage host-vector systems.

As a futher step in evaluating the biologic activity of the polyoma-plasmid and polyoma-lambda recombinant DNA host-vector systems, the scientist tested the ability of the EK2 systems containing polyoma DNA to induce tumors in suckling hamsters.

These animals are highly sensitive to tumor induction by polyoma virus and in some cases even subgenomic fragments of viral DNA can induce tumors in them. The results (Science *205;* 1140–1142, 1979) indicated that inoculation of suckling hamsters with 2×10^8 live cells of *E. coli* K–12 strain Chi 1776 carrying the complete genome of polyoma virus in a recombinant plasmid, failed to induce tumors in any of 32 recipients. Also, lambda phage DNA and particles with a monomeric insert of polyoma DNA did not induce tumors. Purified recombinant plasmid DNA, as well as phage particles and DNA containing a head-to-tail dimer of polyoma DNA, showed a low degree of oncogenicity, comparable to that of polyoma DNA prepared from mouse cells. These findings support the previous conclusions, based on infectivity assays in mice, that propagation of polyoma virus DNA as a component of recombinant DNA molecules in *E. coli* K–12 reduces its biologic activity many orders of magnitude relative to the virus itself.

Currently both the infectivity and tumorigenicity experiments are being extended. In one, *E. coli* K–12 (EK1) containing recombinant plasmids consisting of two copies of polyoma virus DNA and one copy of pBR322 plasmid DNA are being inoculated into newborn hamsters and weanling mice. It is also planned to test lambdaphage lysogens (*E. coli* K–12 containing one or two copies of a lambda bacteriophage-polyoma virus DNA recombinant integrated into the bacterial chromosome) by inoculation into appropriate animal model systems. Results will be published in the open scientific literature and will be presented in a subsequent update of the Final Plan.

6. NIH scientists are also determining the biological activity of *E. coli* K–12 clones carrying DNA copies of an RNA tumor virus. These studies involve the administration of bacterial preparations containing Harvey sarcoma virus DNA to hamsters. These studies are still in progress and when definitive results have been obtained they will be published in the open scientific literature and will appear in an annual update of the final plan.

7. Although they had not appeared in the previous Final Plan, two additional NIAID contract-supported projects are peripherally related to the total risk assessment activities.

a. RAC had suggested that a model cloned DNA segment be constructed to serve as a uniform heterologous DNA segment for risk assessment studies. This endeavor has been successfully completed by an investigator at the University of Wisconsin and employed, as a source of DNA, the plasmid NR79. The EcoRI fragment NR79–R1–G1 contains genes encoding for chloramphenicol, kanamycin and sulfonamide resistance, as well as the promoters that govern these resistances. NR79–R1–G1 was shown not to have an internal EcoRI site and also not to be capable of replicating by itself or with the help of other plasmids. Experimental results indicated that NR79–R1–G1 is not capable of autonomous replication and does not contain an origin of replication that can function in a recombinant plasmid. The antibiotic resistances encoded for by NR79–R1–G1 were tested

and found not to be transposable, that is gene movement and insertion at a new site by a mechanism which does not involve the homologous recombination system of the host strain.

Since the fragment is not capable of autonomous replication, plasmid pJT353, a recombinant plasmid containing NR79–R1–G1 cloned into the EcoRI site of pBR322, is the suggested way of propagating this fragment. Stocks of *E. coli* strain KH802 containing pJT353 have been preserved for the propagation and isolation of NR79–R1–G1. Protocols used for the isolation of purified plasmid pJT353 (NR79–R1–G1 and pBR322) and purified NR79–R1–G1 fragment are available.

b. A contract has been awarded to the University of Minnesota to develop a Comprehensive Course on Microbiological Principles and Techniques for Work with Potentially Biohazardous Agents Including Recombinant DNA. The Principal Investigator will work with the Board of Education and Training of the American Society for Microbiology (ASM) in developing the materials.

In 1977, the ASM undertook a study to devise standards of training for recombinant DNA research workers. A proper state of training of the laboratory workers is the first line of containment and it was their opinion that such training standards should be set by knowledgeable professionals. The product of that study was not standards but rather an outline of a body of knowledge which it was felt that any Principal Investigator should be aware of before independently embarking on recombinant DNA research. This contract will develop all necessary resources to present a course based on the ASM findings.

The plan is for the NIAID to support the development of the resource materials and once that phase is completed, the NIH Division of Safety will have the materials reproduced and distributed to the various Institutions where the NIH supports research to assist them in performing their own local training responsibilities. We will devise both a standard lecture/laboratory course and self study aides.

Eukaryotic Host-Vector Systems

Lower eukaryotic and higher eukaryotic host-vector systems were given a lower priority in the Final Plan than prokaryotic systems.

After discussion at both the March and June 1980 meetings, the RAC recommended modifying the Guidelines to include *Saccharomyces cerevisiae* host-vector systems under section III–O. During their consideration the RAC considered information that: (1) *S. cerevisiae* is nonpathogenic, (2) it does not implant in the intestine, (3) the dilute conditions in which it is found in nature are extremely unfavorable for mating, (4) it does not efficiently compete with wild strains of *S. cerevisiae*, (5) it is fully sensitive to autoclaving and disinfection by standard agents, and (6) mammalian genes cloned in *S. cerevisiae* are not expressed.

RAC also considered the question of proper physical containment for higher eukaryotic viral vectors. The Guidelines have been modified to permit work at more relaxed levels of physical containment that previously required.

The Guidelines have been modified to permit recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) to be propagated and maintained in cells in tissue culture using P1 containment.

It must be shown that the cells lack helper virus for the specific families of the defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one family but each fragment must be less than two thirds of a genome.

No initiatives have been started to directly answer the issues raised in the Final Plan relative to eukaryotic host-vector systems. NIH will continue to monitor the results of free ranging research to collect useful data that can be analyzed as part of the risk assessment process.

III. Implementation

The Final Plan stated that NIAID would recruit and appoint an eminent scientist as a Special Assistant to the Director, NIAID for Risk Assessment. Attempts to recruit a person to fill this position were made including national advertising. No individual with the desired level of credentials and broad recognition by the various scientific disciplines was identified who was willing to undertake this work at the NIH under the conditions we could offer. By mid-year it had become evident that the probable scope and number of various activities encompassed by the Risk Assessment Plan would probably stabilize at the current level and not increase further. Consequently the necessity for a Special Assistant was reconsidered and alternatives for satisfying the needs were evaluated. A decision was made to distribute the proposed functions of the Special Assistant to the Office of Recombinant DNA Activities and the Office of Specialized Research and Facilities, both within the NIAID. When appropriate these offices will use ad hoc consultants in fulfilling the tasks originally described for the Special Assistant and for which staff and the RAC Risk Assessment Subcommittee feel that additional or specific expertise is required.

The remainder of the Implementation section of the plan remains in effect as stated. Most implementation actions have been cited earlier in this document, however, an important part of the Plan are periodic reports to the RAC. In this regard, the Director, NIAID reports to the RAC at each meeting on activities related to risk assessment and provides thereby an important and continuing review of progress and receives the advice or comment of this advisory group.

Dated: September 8, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.*

Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that Circular.

[FR Doc. 80–28453 Filed 9–16–80; 8:45 am]

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